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THE ACTION OF D-TUBOCURARINE AND OF DECAMETHONIUM ON RESPIRATORY AND OTHER MUSCLES IN THE CAT

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In a previous paper (Paton & Zaimis, 1949*b*) we have referred to the way in which decamethonium and D-tubocurarine chloride, when administered intravenously to the cat anaesthetized with chloralose, differed considerably in the readiness with which they depressed the activity of the respiratory muscles relative to that of the limb muscles. This difference has been further studied in the experiments now described, of which a brief account has already appeared (Paton & Zaimis, 1949*a*).

The mode of action of decamethonium has recently been further analysed (Brown, Paton & Vianna Dias, 1949; Burns, Paton & Vianna Dias, 1949; Zaimis, 1949; Buttle & Zaimis, 1949). We may conclude that the main difference between decamethonium and D-tubocurarine is the following. D-Tubocurarine paralyses neuromuscular conduction by rendering the end-plate less sensitive to the depolarizing action of acetylcholine. Decamethonium, however, like acetylcholine, depolarizes the end-plate region, and may, like acetylcholine, according to the circumstances either initiate a propagated contraction in the muscle, or give rise to neuromuscular block. Decamethonium should thus be compared with acetylcholine rather than with curare in its mode of action. This fundamental distinction from curare needs to be borne in mind in considering the differences, described below, between the two drugs.

METHODS

Cats anaesthetized with chloralose (80 mg./kg.), after induction by ether, were used in most experiments. Twitches and tetani of the soleus and tibialis muscles were excited by maximal shocks applied to the tied sciatic nerve in the thigh. The contractions of these muscles were applied to a flat spring myograph and recorded on a smoked drum. In some experiments records of the mechanical response of the two muscles in the same leg were recorded on the same drum.

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The respiration was recorded either by discharging the respirations by means of light rubber valves into a large reservoir fitted with a fine leak, the pressure within which was recorded by a sensitive tambour; or by the respiration recorder described by Paton (1949). Simple mechanical records of the respiratory movements were found to give little indication of the true pulmonary ventilation.

Phrenic nerve action potentials were recorded from the central stump of a cut phrenic root, close to its origin in the neck. The root was dissected as required. The potential changes were amplified on to a screen of a cathode-ray oscillograph, and photographed on moving paper. Action potentials from the diaphragmatic muscle were recorded in the same way from two silver electrodes about 1 cm. apart insulated to their tips and placed in line along a suitable bundle of muscle fibres on the abdominal surface of the diaphragm. Bronchoconstriction was measured as described in the text. Decamethonium was always used as the iodide, and D-tubocurarine as the chloride.

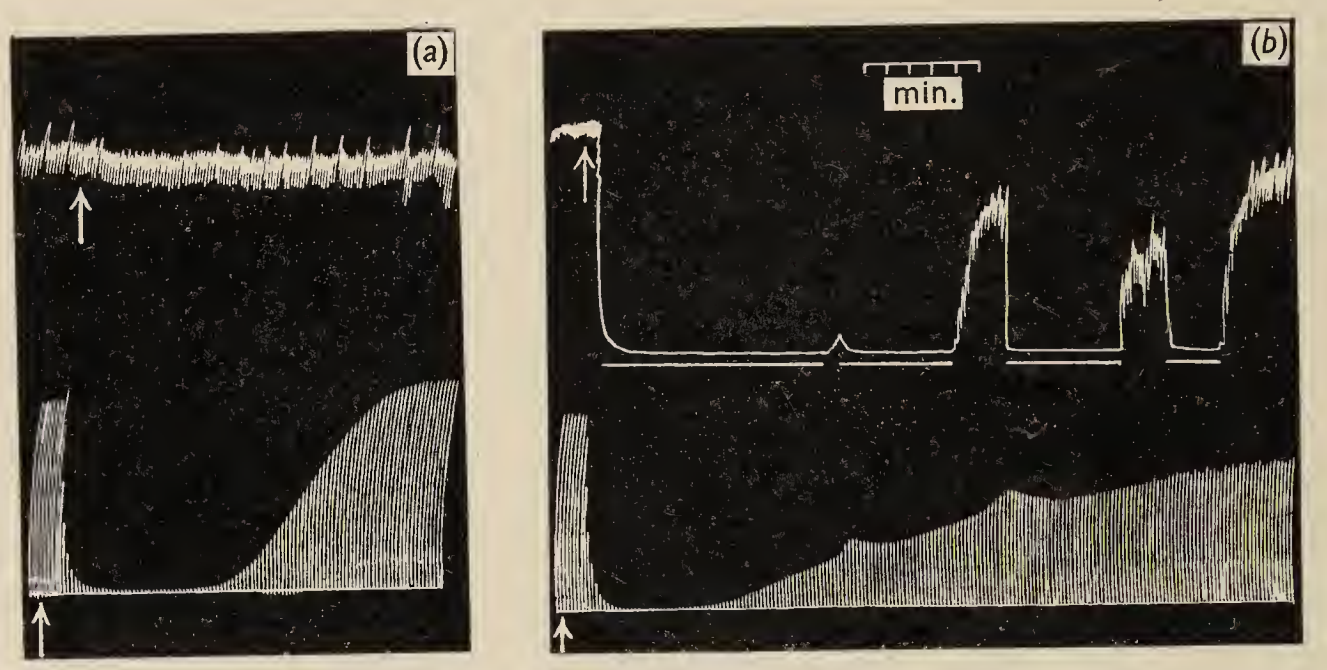


Fig. 1. Cat. Chloralose. Record of respiration and of contractions of tibialis excited by maximal shocks to the sciatic nerve every 10 sec. (*a*) At arrow, decamethonium iodide 60 μg. intravenously. (*b*) At arrow, D-tubocurarine chloride 1 mg. intravenously. Artificial respiration applied during white horizontal line.

RESULTS

Cats under chloralose anaesthesia

Fig. 1 illustrates typical effects of decamethonium iodide (60 μg.) and of D-tubocurarine chloride (1 mg.) on respiration and on twitches of tibialis excited maximally through its nerve. Both drugs produce in this experiment almost complete neuromuscular block in tibialis. Respiration is not affected by decamethonium; but, after D-tubocurarine the respiration is completely paralysed, and remains inadequate for 20 min., although during this time the twitch of tibialis has recovered almost completely.

Effects by decamethonium on respiration can, nevertheless, be demonstrated. Doses sufficient just to paralyse tibialis (approx. 30 μg./kg.) occasionally cause a transient depression of 10–15% in the respiratory minute volume; the effect passes off in 2–3 min., long before the effect of tibialis twitch has disappeared. With larger doses more severe respiratory depression is obtained, and may be complete with a dose of 70–80 μg./kg. Even in these experiments, however

the effect on the respiration passes off earlier than the effect on the tibialis muscle. Thus in one experiment, after an intravenous injection of 260 μg./kg. of decamethonium iodide, respiration returned to normal within 30 min., although it took more than 1 hr. 20 min. before the tibialis muscle had recovered.

The quantitative difference in the actions of decamethonium and of D-tubocurarine is also shown by choosing doses which produce approximately the same moderate degree of respiratory paralysis. In Table 1 are given for each drug the dose which just begins to affect the respiration significantly, and the dose which causes 95% paralysis of the tibialis twitch. The doses are mean values from fifteen experiments.

TABLE 1. Comparison of doses of D-tubocurarine or of decamethonium required either to paralyse tibialis twitch 95%, or to begin to depress respiration, under chloralose or under ether anaesthesia.

	For paralysis of tibialis		For depression of respiration	
	Chloralose (mg./kg.)	Ether (mg./kg.)	Chloralose (mg./kg.)	Ether (mg./kg.)
D-Tubocurarine chloride	0·4	0·27	0·08	0·04
Decamethonium iodide	0·035	0·07	0·04	0·04

One of the differences between decamethonium and D-tubocurarine is the fact that paralysis by decamethonium is preceded by an initial stimulating action which D-tubocurarine lacks. As seen in Figs. 1–3, decamethonium produces, on the tibialis muscle, an augmentation of the twitch, or even spontaneous contractions, before the onset of paralysis. This effect is less pronounced on the respiration, but sometimes decamethonium, especially when given in small doses, produces a slight augmentation of the amplitude of the respiratory movements, so that the minute volume increases. This is never observed with D-tubocurarine.

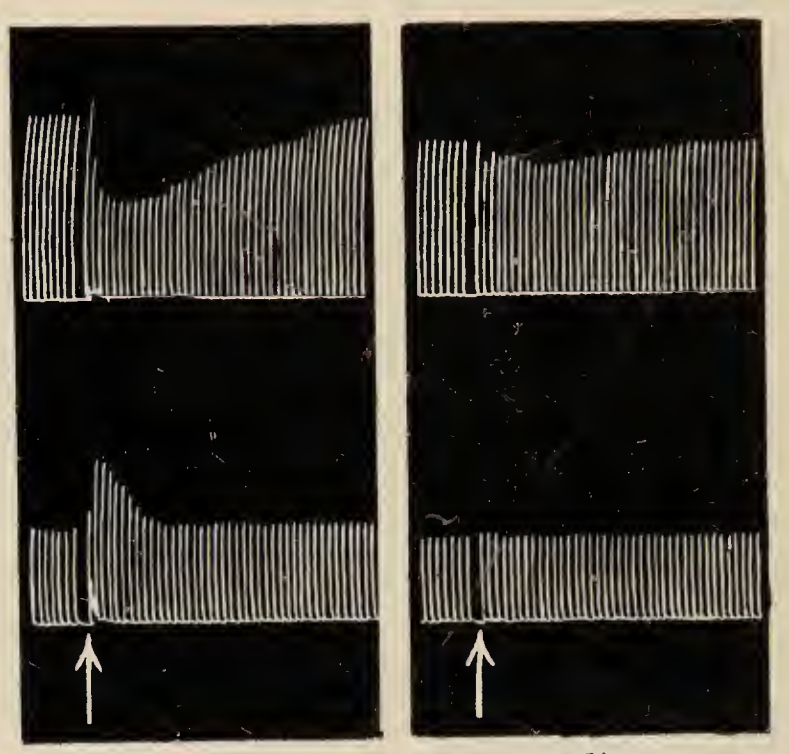
When the tibialis muscle is paralysed by D-tubocurarine, and artificial respiration is stopped at a time when natural respiration is still inadequate, there occurs a slight transient decurarization (Fig. 1). This effect has never been observed with decamethonium. We have not analysed the cause of this decurarization, or of the absence of a similar phenomenon with decamethonium. Experiments on the effects of adrenaline on muscles paralysed by D-tubocurarine or decamethonium suggest that a discharge of adrenaline from the suprarenals is inadequate to account for the phenomenon.

Antagonism to decamethonium by pentamethonium

We have described elsewhere the action of pentamethonium (the pentane homologue of decamethonium) as an antagonist to the muscular and respiratory paralysis by decamethonium. Fig. 2 exemplifies the antagonism. In this experiment a dose of 30 μg./kg. decamethonium iodide caused, after an initial period of augmentation of the twitch tension, a rapidly progressing paralysis of the tibialis twitch; a second identical dose of decamethonium some hours

later led to complete neuromuscular block lasting more than 20 min. But 2 min. after the first dose the paralysis was checked and reversed by the injection of 10 mg. pentamethonium iodide. The respiratory minute volume was reduced by both doses of decamethonium to about 40 % of its initial value; but as soon as pentamethonium was given, the minute volume promptly rose again to its original height and beyond. This 'overswing' in the recovery of the

Fig. 2.



(*a*) (*b*)

Fig. 3.

Fig. 2. Cat, 3·1 kg. chloralose. Respiration and tibialis twitch. At first arrow, decamethonium iodide 90 μg. intravenously; at the second arrow, pentamethonium iodide 10 mg. intravenously.

Fig. 3. Cat, 3·8 kg. chloralose. Contractions of tibialis (above) and soleus (below) to maximal nerve shocks. (*a*) Chloralose anaesthesia; at arrow, 20 μg. decamethonium iodide into iliac artery. (*b*) After 1 hr. ether anaesthesia; at arrow, 20 μg. decamethonium iodide into iliac artery.

respiration is a common phenomenon when pentamethonium is given after a paralysis by decamethonium; it often makes the antagonism of pentamethonium to decamethonium appear much more effective on the respiration than on the muscle twitch. It probably results from an increase in activity of the respiratory centre while respiration is depressed, such as we describe below; only when the weakness of the respiratory muscles is removed by pentamethonium can this increased activity of the centre be revealed in a period of hyperventilation.

Ether anaesthesia

With ether anaesthesia higher doses of decamethonium are required to abolish the twitch of tibialis than with chloralose anaesthesia, but no similar increase in dose is required to paralyse respiration (Table 1). Thus, under ether a dose

of 60 μg./kg. which depresses the tibialis twitch by 40–70 % will affect the respiratory movements to an equal degree, and the characteristic difference observed in chloralose anaesthesia between the actions of decamethonium on tibialis twitch and on respiratory movements is less pronounced.

The augmentation of respiratory movements observed occasionally after small doses of decamethonium in cats under chloralose was never seen under ether anaesthesia. This corresponds to our previous observation that under ether the potentiation of the muscle twitch does not occur. Fig. 3 illustrates the abolition by ether of the potentiation of the twitch of tibialis due to decamethonium, together with the diminution of its paralysing action. In this experiment, the twitches of the tibialis were recorded simultaneously with those of the soleus muscle. The stimulating action of decamethonium on this latter muscle is more pronounced than on the tibialis, but again it is abolished by ether.

Whereas in ether anaesthesia, the threshold dose of decamethonium for affecting the tibialis was raised, the reverse was true for D-tubocurarine (Table 1). Paralysis of the tibialis twitch was obtained with a dose of D-tubocurarine approximately 30 % less than that required in cats anaesthetized with chloralose. In addition, D-tubocurarine also became more active in depressing respiration; the threshold dose required to do this was reduced to 0·04 mg./kg., about half that required in chloralose anaesthesia. Even under ether anaesthesia, therefore, decamethonium still affects the respiration relatively less than does D-tubocurarine.

Unanaesthetized animals

Actions in monkeys

A comparison was made, for both drugs, of the dose required to cause general muscular weakness with that required to produce impairment of respiratory movements. The muscular weakness was assessed in monkeys by their inability to sit up or to support their heads or arms, and by infrequency and weakness of spontaneous movements. In each experiment the time required for complete recovery from paralysis was determined. In assessing impairment of respiratory movements, depression of the respiration so far as to necessitate artificial respiration was chosen as a convenient reference point. The results of repeated tests on six monkeys are given in Table 2.

The injection of 0·5–0·77 mg./kg. decamethonium caused general motor paralysis lasting 55–130 min. Artificial respiration was needed in only 17 % of the animals receiving 0·5 mg./kg. and in 80 % of those receiving 0·77 mg./kg. decamethonium iodide. On the other hand, 0·15 mg./kg. of D-tubocurarine necessitated artificial respiration in all the animals tested, although recovery of motor paralysis was complete in 30 min. The predilection of D-tubocurarine for respiratory effects in the monkey was particularly striking with small doses; even 0·075 mg./kg. caused exaggerated movements of the nostrils and thorax

suggestive of respiratory distress, without eliciting any signs of paralysis of postural muscles or of reduction of motor power.

The results of Table 2 show further that threshold motor paralysis is obtained with a dose of decamethonium slightly smaller than 0·3 mg./kg.; but to produce in 50% of the animals respiratory depression requiring artificial ventilation, a dose of over 0·6 mg./kg. is needed; the ratio of the latter dose to the former is estimated to be about 2·6:1. For D-tubocurarine the corresponding doses were about 0·09 and 0·12 mg./kg., with an estimated ratio of 1·3:1.

TABLE 2. Comparison of respiratory paralysing effects of decamethonium and D-tubocurarine chloride in the unanaesthetized monkey and cat

Compound	Dose (mg./kg.)	Number requiring A.R. / Number tested	%	Mean time in min. for full recovery of muscle strength
		Monkey		
Decamethonium iodide	0·3	0/5	0	16
	0·5	1/6	17	55
	0·6	2/6	33	—
	0·77	4/5	80	130
D-Tubocurarine chloride	0·075	0/5	0	3
	0·10	0/5	0	25
	0·125	2/3	67	—
	0·150	3/3	100	29
		Cat		
Decamethonium iodide	0·03	0	0	10
	0·04	0	0	14
	0·05	0	0	30
	0·06	3	100	31
D-Tubocurarine chloride	0·2	1	25	$9\frac{1}{2}$
	0·25	2	100	17

A.R. = Artificial respiration.

Actions in cats

Similar observations were made in cats, but it was not possible to do more than a few experiments, because the usual means of applying artificial respiration (manual compression of the chest; Eve's method adapted to cats; or intermittent positive pressure applied through a face mask) were found to be rather ineffective. Our conclusions, therefore, take into account the individual behaviour of each cat used. With decamethonium, motor paralysis lasting for about 10 and 30 min. was produced by 0·03 and 0·05 mg./kg. of decamethonium respectively. These doses did not impair respiration sufficiently to enforce artificial respiration, and respiration seemed quite unaffected with doses of 0·03 mg./kg. Increasing the dose to 0·06 mg./kg. was, however, enough to depress the respiration seriously. With D-tubocurarine, even at 0·2 mg./kg., appreciable respiratory depression was present; increasing the dose only to 0·25 mg./kg. increased the respiratory depressant action to a dangerous level, although the paralysis still lasted little more than $\frac{1}{4}$ hr. We have again estimated the ratio of the respiration-paralysing dose to the threshold motor paralysing dose; for decamethonium iodide this was about 2:1, and for D-tubocurarine about 1·2:1.

Actions in rabbits

Motor paralysis in the rabbit was measured by the failure of the righting reflex. Doses of decamethonium as well as of D-tubocurarine (given in single rapid intravenous injections), which just produced such failure, had to be increased by about 20% in order to bring about a condition of respiratory distress requiring artificial ventilation. The two drugs did not, in these experiments, show the characteristic differences seen in cats and monkeys. During experiments with the slow infusion head-drop method, on the other hand, this difference appeared to become noticeable; even then the difference between decamethonium and D-tubocurarine remained slight and was certainly not as pronounced as in cats and monkeys.

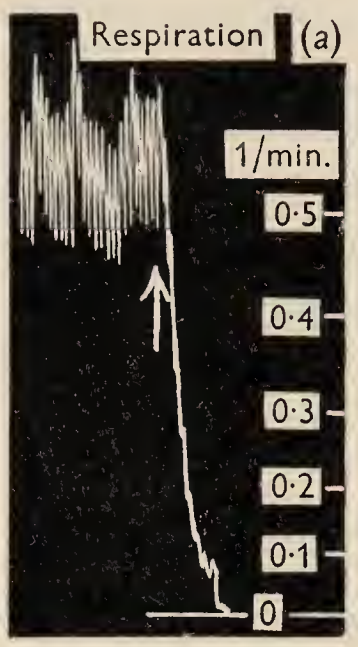
Absence of depression of the respiratory centre by D-*tubocurarine*

The sequence of events during the development of respiratory depression due to D-tubocurarine indicates clearly that paralysis of the respiratory centre is not the cause of its relatively strong action as respiratory depressant. The decrease in the amplitude of the respiratory movements is associated with a widening of the range of muscles participating in respiration. Thus, in cats under chloralose the slow and full respiratory movements of diaphragmatic origin change progressively after D-tubocurarine into movements involving more and more other thoracic and abdominal muscles. The frequency of respiration sometimes increases, but it does not decrease. Widespread convulsive attempts finally supervene, but even then the movements remain feeble. Ultimately, respiration assumes a gasping character.

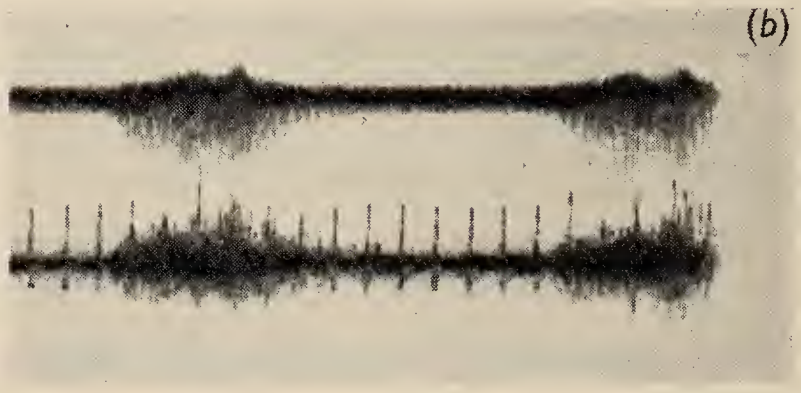
The general picture is the same in the unanaesthetized monkey. Again the quiet respiration gives way, after D-tubocurarine, to distinct thoracic and abdominal efforts, with dilatation of the nares at each inspiration and an irregular acceleration of respiration. Finally, there are gasping movements of feeble character, which, however, involve all the auxiliary respiratory muscles. Such a response is quite different from that seen after a central respiratory depressant, like thiopentone or morphine, which cause slowing of respiration and inactivity of the auxiliary respiratory muscles. The respiratory impairment after D-tubocurarine differs also from the ordinary response to asphyxia in which the auxiliary muscles are thrown into violent and powerful activity.

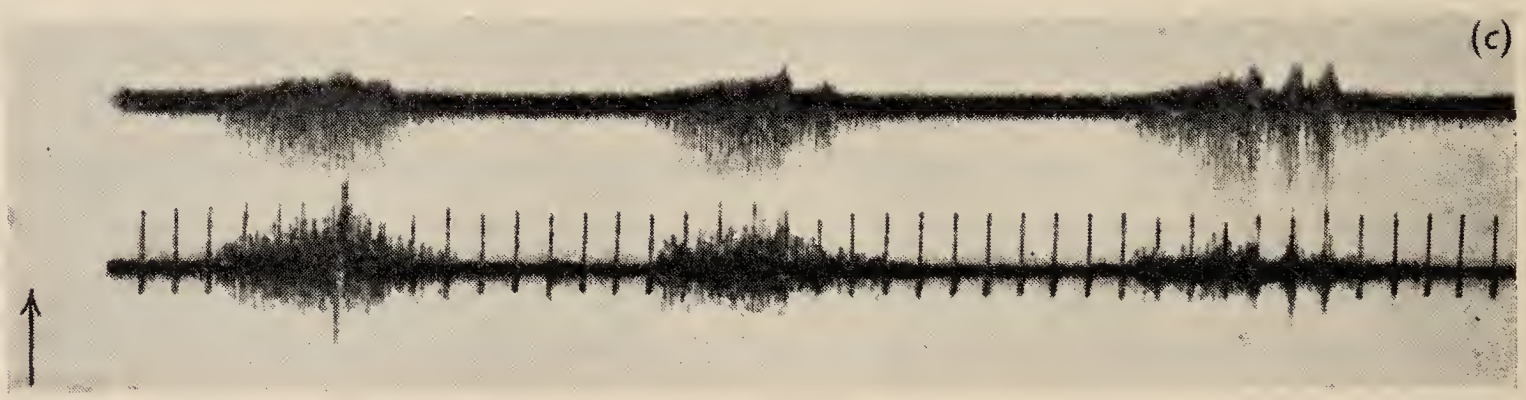
Direct evidence for the absence of any central respiratory depression after D-tubocurarine has been obtained by recording in a cat the action potentials from the cut central stump of a phrenic nerve root. In the experiment of Fig. 4 these action potentials were recorded simultaneously with those from the diaphragm and with the respiratory minute volume. The injection of 0·72 mg. D-tubocurarine produces rapid abolition of pulmonary ventilation, as shown from the record of the respiratory minute volume. The muscle action potentials

diminish rapidly; even in the third breath after the injection a considerable reduction has occurred. The action potentials of the phrenic nerve root, however, show no sign of diminution at any time; indeed, the first change consists



Before injection





0·72 mg. D-tubocurarine

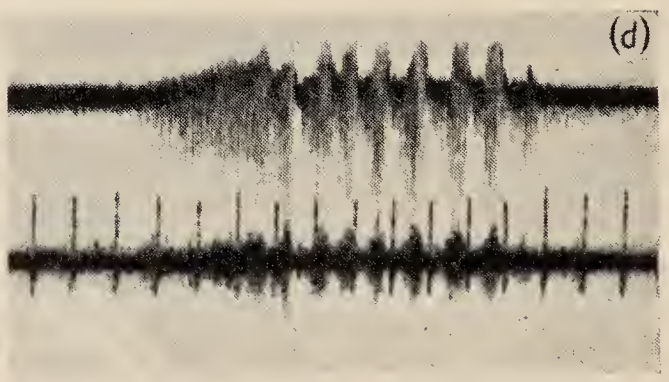


Fig. 4. Cat, 3·2 kg. chloralose. (*a*) Record of respiratory minute volume; injection of D-tubocurarine chloride 0·72 mg. intravenously. (*b*)–(*d*) Action potentials of central slip of phrenic root (above) and action potentials from diaphragmatic muscle (below); (*b*) two breaths immediately before injection of D-tubocurarine; (*c*) 1st, 2nd, 3rd; and (*d*) 10th breaths after injection of D-tubocurarine. (Note electrocardiograms superimposed on diaphragm muscle action potentials.)

in a prolongation and intensification of the discharge during each respiratory movement. Records taken from preparations of the phrenic nerve root containing two or three active nerve fibres showed that the intensification of the

discharge is partly accounted for by an increase in the frequency of the discharge in the fibres; after an injection of 0·48 mg. D-tubocurarine intravenously, this frequency rose from 10 to 15/sec. during normal respiration to a peak value of 40/sec., at the same time as the respiratory minute volume fell by 60%.

It is interesting to notice, in the record of the nerve action potentials in Fig. 4, characteristic fluctuations after D-tubocurarine. Before the injection of this drug the respiratory discharge rises and falls more or less smoothly during each inspiration. By the third breath after the injection there are three pronounced fluctuations within the single inspiratory discharge, and by the 10th breath there are nine such fluctuations, rather closely related in time to the pulse rate. We did not analyse the cause of these rapid oscillations in the respiratory discharge; but whatever their cause, they do not suggest any depression of the respiratory centre.

Similar results from the phrenic nerve preparation were obtained after decamethonium. No alteration in the respiratory discharge could be discerned until it began to increase with asphyxia. The differences between decamethonium and D-tubocurarine cannot, then, be ascribed either to central depressant action by D-tubocurarine, or to a central stimulant action by decamethonium.

Salama & Wright (1950) have shown in the cat that direct intracisternal injection of 0·4 mg. D-tubocurarine chloride will stimulate the rate and depth of respiration, together with the other medullary centres. But we are doubtful whether so small a dose as 0·48 mg. given intravenously could possibly imitate these effects on the respiration. It certainly fails to reproduce the effect of an intracisternal dose on the blood pressure, described by these authors; and there is, in addition, evidence that the drug penetrates the blood-brain barrier with difficulty. Adrian & Bronk (1928) showed that asphyxia caused an intensification of respiratory discharge such as we have described; since, in our experiments above, the respiratory minute volume was rapidly and substantially depressed, and this depression was always accompanied by a proportionate increase in the electrical activity recorded from the phrenic nerve, we believe that this increase was, in fact, due to the accompanying asphyxia.

Absence of obstruction to respiratory air flow

D-Tubocurarine causes no obstruction to the flow of air in and out of the lungs. This possibility had to be considered, particularly because of its histamine-liberating property. Indeed, if the release of histamine were associated with respiratory depression, the difference in the effectiveness of decamethonium and D-tubocurarine could be explained by this action, since D-tubocurarine is a stronger histamine liberator than decamethonium (MacIntosh & Paton, 1949).

It was found, however, that neither histamine nor propamidine, which is an even stronger histamine liberator than D-tubocurarine, depress respiration; in cats under chloralose, in fact, these substances sometimes increase the respiratory minute volume, probably through a reflex mechanism, responding to the fall in arterial blood pressure.

The first method by which the effect of D-tubocurarine on the air flow was measured was by recording simultaneously the respiratory minute volume and the intrapleural pressure. This is a simple and convenient technique, which has the advantage of retaining natural respiration. The volume of air moved in each breath is recorded, together with the pressure fluctuation which

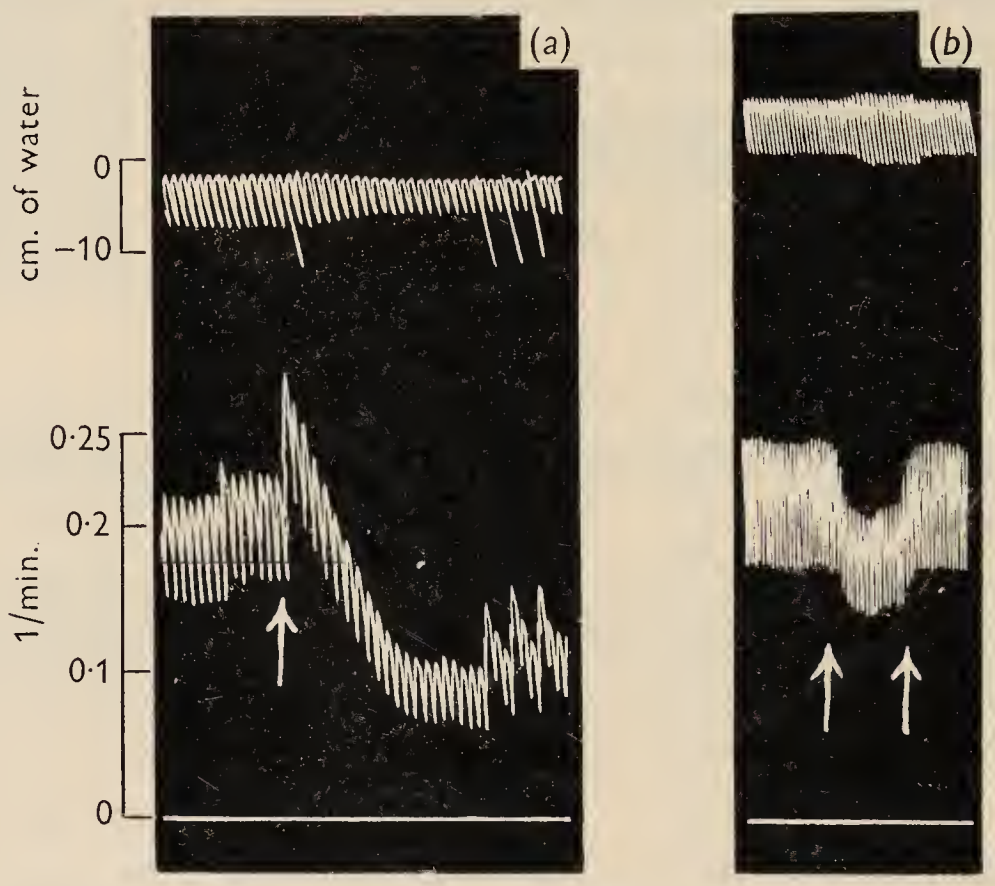


Fig. 5. Cat, 2·2 kg. chloralose. Record of intrapleural pressure and of respiratory minute volume. (*a*) At arrow, injection of 0·3 mg. D-tubocurarine chloride intravenously. (*b*) From another experiment. Trachea partially occluded between arrows.

moved this volume, and by comparing these, we obtain a measure of the pulmonary resistance. Fig. 5*b* illustrates the change in such a record that results from a mechanical obstruction to respiration; the minute volume is depressed, but the intrapleural pressure fluctuation is increased, and hence the resistance to air flow must be increased. After an injection of D-tubocurarine, however, no such effect is observed (Fig. 5*a*). On the contrary, the excursions of intrapleural pressure diminish at the same time and roughly in the same proportion as the minute volume, apart from the exaggerated deflexion observed during gasping, seen for instance at the end of Fig. 5*a*, as asphyxia progresses. The experiment, in fact, offered evidence that the resistance to respiration had remained constant, and that the source of the pressure change (i.e. muscular activity) had been weakened. But the estimate of the resistance of respiration by this means is not accurate enough to exclude the possibility

of a small element of bronchoconstriction contributing to the respiratory depression, masked by a larger degree of neuromuscular block.

A more rigorous test was provided by the use of a pump to force a fixed volume of air periodically into the lungs, recording at the same time the air-pressure in the trachea. We were unable to obtain a significant bronchoconstriction with

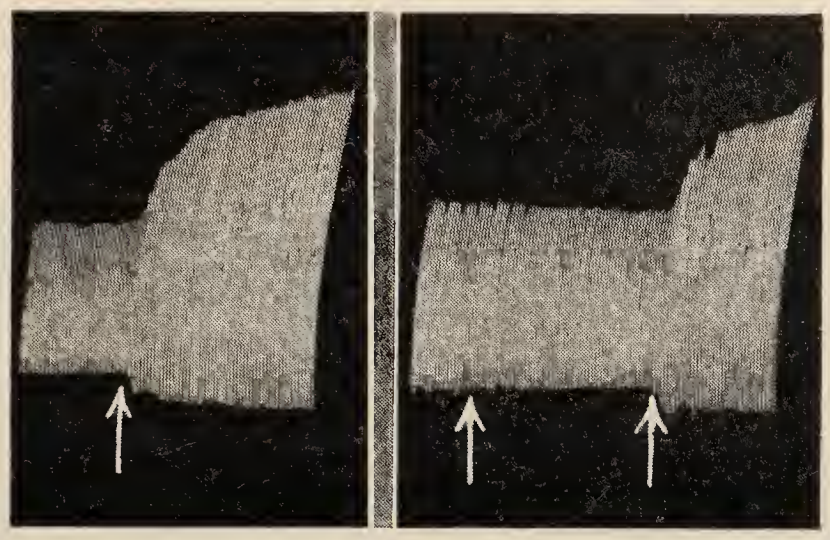


Fig. 6. Cat, 3·1 kg. chloralose. Record of intratracheal pressure during artificial respiration with chest open. (*a*) At arrow, 0·5 mg. arecoline intravenously. (*b*) At arrow, D-tubocurarine chloride 1 mg. intravenously, then at second arrow arecoline 0·5 mg. intravenously.

histamine, but arecoline (0·5 mg. i.v.) elicited a good response (Fig. 6*a*). Injection of D-tubocurarine, however, in a dose of 1·0 mg., was entirely without effect, although the bronchial muscle was shown to be responsive to arecoline immediately afterwards (Fig. 6*b*).

Differences in the neuromuscular block produced by decamethonium and by D-*tubocurarine*

Effect on single contractions and on a tetanus of muscle

It is well known that the muscle curarized by D-tubocurarine chloride is unable to maintain a tetanus at its initial strength. Characteristically, the tension exerted by the curarized muscle during a tetanus is initially comparable to the tension developed during a single twitch, and then rapidly falls to a much lower level which is maintained fairly constant for tetani of durations up to 10 sec. As the dose of curare increases, and as the frequency of tetanization increases, the ratio of the maintained tension to the twitch tension becomes smaller, and often becomes zero. Thus, in a muscle in which the twitch has been reduced by 50%, the tension developed by a tetanus at 50/sec. usually falls rapidly to an undetectable value. Fig. 7 illustrates the phenomenon on cat's tibialis, after a dose of 0·3 mg. D-tubocurarine intravenously, during stimulation of the sciatic alternately with single shocks and with a tetanus at 20/sec. of 5 sec. duration; in this experiment the ratio of the maintained tetanic tension to the twitch tension fell from a normal value of 0·65 to 0·063 at the

deepest point of the block, although the single twitch was not reduced by more than about 40%.

The same phenomenon is also shown in Fig. 7 in another muscle, soleus. This muscle fuses its contractions far more readily than does tibialis; and hence at this frequency of stimulation the tetanus/twitch ratio is initially high, about 7. At the depth of the paralysis by D-tubocurarine chloride, this ratio sinks to about 1, although it is difficult to estimate the ratio accurately with these feeble contractions.

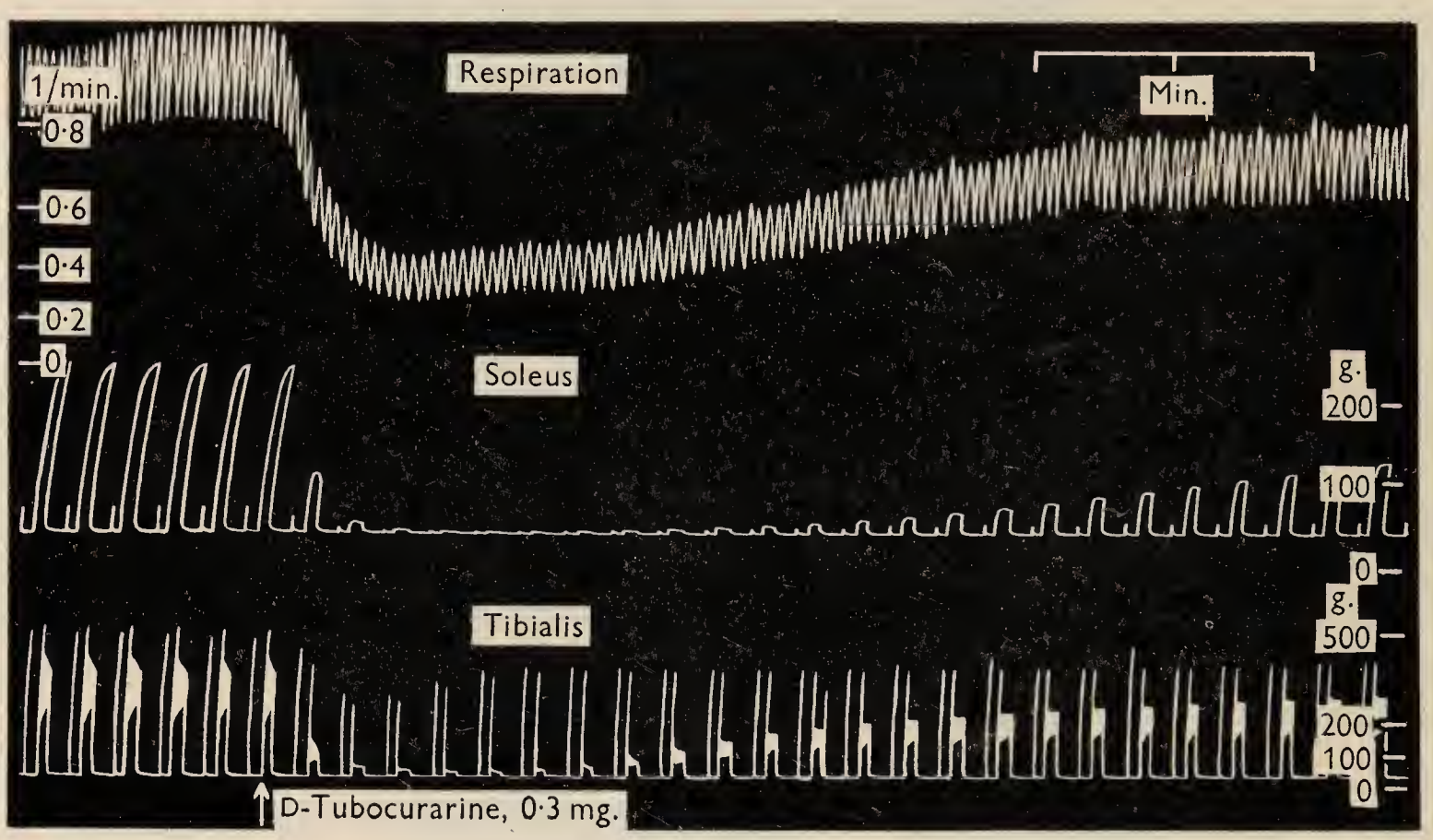


Fig. 7. Cat 2·1 kg. chloralose. Simultaneous records of respiratory minute volume, contractions of soleus, and contractions of tibialis. Soleus and tibialis excited alternately with single maximal shocks and with tetani at 20/sec. to the sciatic nerve. At arrow, D-tubocurarine chloride 0·3 mg. injected intravenously.

During neuromuscular block by decamethonium the results of a similar experiment are different (Fig. 8). On the tibialis muscle, the tetanus/twitch ratio actually rose from 0·67 to 1·6, after a dose of decamethonium sufficient to diminish the tension of single twitches to less than one-fifth of their initial value. The paralysis of soleus is not sufficient to allow the calculation of similar ratios; but it is clear that the proportionate depression of the maintained tetanic tension is not greater than that of the twitch.

A comparison of the first four twitch/tetanus pairs, in Fig. 8, after the injection of decamethonium, illustrates a significant point, that the tetanus/twitch ratio in the tibialis muscle treated with decamethonium increases as block deepens. This finding will be discussed later in connexion with the compound character of the tibialis.

The comparison of decamethonium and D-tubocurarine depends to some extent, therefore, on whether single contractions of a muscle or a tetanic activity of a muscle are used as test object. When tested on single twitches, decamethonium has its highest potency relative to D-tubocurarine. But its relative potency falls when the comparison is made using intermittent tetani; decamethonium remains about equally active, but D-tubocurarine may then appear twice as effective as before. We have, then, one important mechanism contributing to the relative sparing of respiration by decamethonium; for

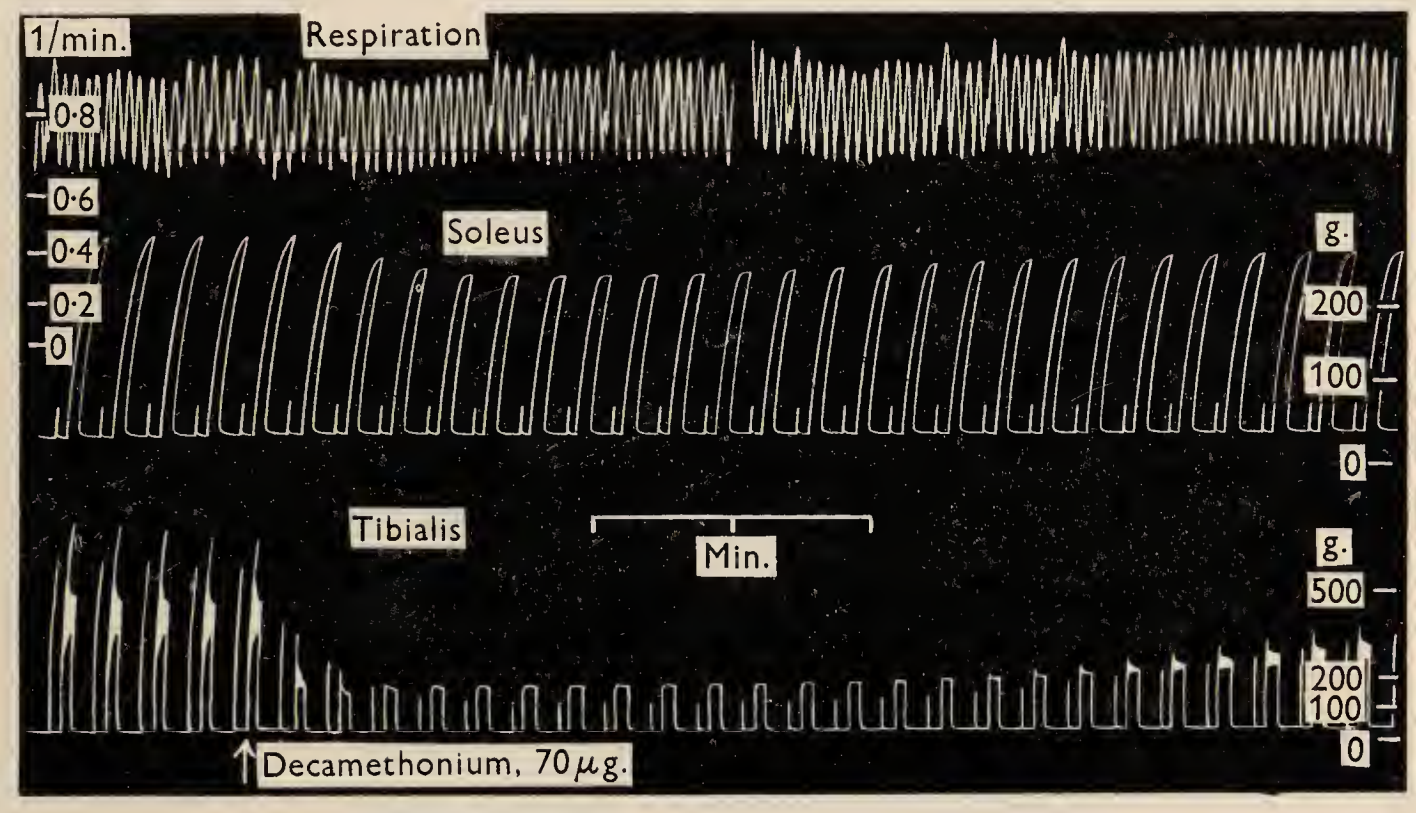


Fig. 8. As Fig. 7. At arrow, decamethonium iodide 70 μg. injected intravenously.

respiration is a tetanic activity, consisting of intermittent periods of stimulation of the diaphragmatic muscle fibres at a frequency of about 20/sec. (Adrian & Bronk, 1928). But this does not explain how decamethonium can paralyse respiration less than the muscular activity involved in posture and movement, for such activities are also tetanic in nature.

Comparison between effects on tibialis and on soleus muscles

In Fig. 7 the dose of D-tubocurarine was sufficient to paralyse both twitches and tetani of soleus almost completely; but the twitch of tibialis was only depressed about 50%, and even the tetanus of tibialis still possessed 10% of its original strength. We have observed this relative sensitivity of soleus every time the comparison has been made. Taking all the experiments into account, we estimate that it needs about 50–70% more D-tubocurarine to paralyse twitches of tibialis fully than to do this to soleus.

Fig. 8 shows the contrast presented when decamethonium is injected. It is now tibialis which is deeply paralysed, the twitch being reduced to 20% and the tetanus to 30% of normal, although the contractions of soleus are little

depressed. This, too, is a regular phenomenon, and we have been able again to estimate the relative sensitivities of the two muscles; it needs about 30–50% more decamethonium to paralyse soleus than to paralyse tibialis.

These differences do not represent a variation in the amount of injected drug reaching the two muscles. The experiments with simultaneous recording from the two muscles were undertaken so that the comparison between the muscles should be made while each was exposed to the same concentration of paralysing drug in the blood. But it is conceivable that the rates of blood flow in each muscle, and hence of uptake of a drug, might differ. This cannot have affected the results, however, because the exposure to the drug lasts for a considerable time, a period many times longer than the circulation time (cf. Gray & Paton, 1949). Further, the time of onset of paralysis and the time of maximum effect would have been different in the two muscles, if differences in speed of reaching equilibrium with the circulating drug had existed; in fact, however, these times were always the same for the two muscles. The differences in depth of paralysis must, therefore, be due to differences in sensitivity to the drug.

Figs. 7 and 8 include records of the respiratory minute volume. In Fig. 7 it is not possible to say whether the respiratory muscles are following soleus or tibialis the more closely, since the depression of the tetanus by D-tubocurarine is substantial in both muscles. But in similar experiments, with slightly smaller doses, we have seen depression of the respiration where neither twitch nor tetanus of tibialis were much affected, but soleus was considerably blocked. In Fig. 8, after an injection of decamethonium, by which twitches and tetani are almost equally paralysed, there is still clearer evidence that soleus parallels the behaviour of the respiratory muscles; for although tibialis is 80% paralysed, soleus is hardly affected, and the respiration not at all.

Our results establish, therefore, that tibialis and soleus differ considerably in their sensitivity to decamethonium and to D-tubocurarine, and that of these two muscles, soleus resembles the respiratory muscles much more closely than does tibialis in its response to these drugs.

Sensitivity of the diaphragm

It follows from what has been already described, that the diaphragm should be more sensitive to D-tubocurarine than to decamethonium. We are greatly indebted to Dr Wien for testing this point for us on the isolated rabbit and kitten diaphragms *in vitro*. Using single shocks at a rate of 8/min., he found that on the rabbit diaphragm, decamethonium was one-quarter as active as D-tubocurarine; and on the kitten diaphragm, one-half as active as D-tubocurarine. Since, with the rabbit head-drop test decamethonium is roughly twice as active as D-tubocurarine, and with tibialis twitch in cat about fifteen times as active, it is clear that in these species the diaphragm is relatively resistant to decamethonium.

The respiratory depressant action of D-*tubocurarine after prostigmine*

The fact that decamethonium has a relatively weak action on the respiration in comparison to D-tubocurarine is not to be attributed to the weak anticholinesterase activity of decamethonium which D-tubocurarine does not share. For, if D-tubocurarine is tested in the presence of an anticholinesterase such as prostigmine, it retains its respiratory depressant action. In the experiment of Fig. 9, a dose of prostigmine was given which produced a large potentiation of the tibialis twitch and raised the paralysing dose of D-tubocurarine to more than

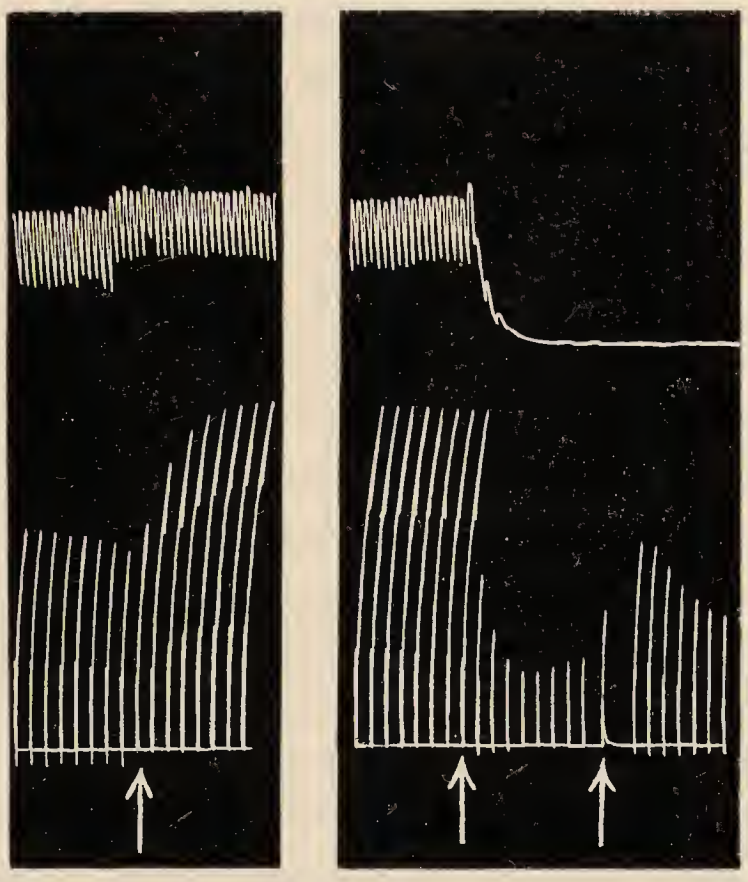


Fig. 9. Cat, chloralose. Record of respiratory minute volume and of contractions of tibialis; nerve shocks every 10 sec. (*a*) At arrow, injection of 0·5 mg. atropine sulphate and 1·25 mg. prostigmine methylsulphate. (*b*) At arrow, injection of D-tubocurarine chloride, 2·5 mg. At second arrow, tetanus at 50/sec. for 5 sec.

twice its original value. But, despite this clear evidence of successful antagonism to the muscle cholinesterase, a subsequent dose of D-tubocurarine which only paralysed the tibialis twitch about 40 %, caused complete, rapid and sustained paralysis of respiration.

DISCUSSION

Effects on the respiration

The experiments of this paper started by comparing the effects of decamethonium and D-tubocurarine on the respiratory muscles and on the tibialis twitch. In the cat under chloralose, the respiration is paralysed by D-tubocurarine before, and by decamethonium after, the twitch of the tibialis. This difference is not due to depression of the activity of the respiratory centre by D-tubocurarine, nor to interference by this drug with the mechanical task of respiration; nor is it due to the weak anticholinesterase activity possessed by decamethonium. But there

is a real difference in the neuromuscular blocks produced by the two compounds.

In analysing this result, it was found that two characteristics in the behaviour of the neuromuscular blocks were of particular significance. First, there is a difference in the effect of decamethonium and of D-tubocurarine on the twitch and on the summated response of a muscle. For instance, D-tubocurarine affects the tetanus of the tibialis more than the single twitch; a dose which only moderately reduces the twitch may abolish the tetanus. This is not so when the muscle is under the influence of decamethonium. Under these conditions the tetanus is well maintained and its tension, even if reduced, remains greater than that of the twitch. We have pointed out (Paton & Zaimis, 1949) that this difference between decamethonium and D-tubocurarine suggests a different mechanism of neuromuscular block, which later experiments have in fact established. It follows from this difference that any comparison of the drugs on a particular muscle will depend on the state of activity of that muscle. In our first experiments the tibialis twitch was compared with the respiratory movements. The fact that the latter are tetanic in character must contribute substantially to the selective depression of the respiration by D-tubocurarine. In general terms, it can be concluded that in muscles paralysed with decamethonium the reduction of a twitch-like or of a tetanic contraction will be more or less the same, whereas under D-tubocurarine the effect will depend to a great extent on the intensity of activity of the muscle.

Secondly, there is a difference in the sensitivity of different muscles to decamethonium and to D-tubocurarine, which may be related to their classification as 'red' or 'white', as will be discussed below. This fact emerged when, in addition to the tibialis, the soleus muscle was investigated. The soleus was found to be more sensitive to D-tubocurarine than was tibialis, and less sensitive to decamethonium. In these respects soleus was paralleled by the respiratory muscles. Our finding of the strong depressant action of D-tubocurarine on the respiration in cats is therefore explained partly because respiration is a tetanic movement, and partly because the reactions of the respiratory muscles appear not to resemble those of tibialis but rather those of the soleus muscle.

It has, however, been claimed that curare paralyses the diaphragm (or the respiratory muscles) last of all the striated muscles (e.g. Tillie, 1890; Gray & Halton, 1946). This appears to contradict our result that the earliest action of D-tubocurarine is a depression of the respiration. There are certain difficulties in comparing experiments of this sort. The preparation of curare, the anaesthetic, the experimental animal, the frequency of stimulation of a muscle, the method of recording respiration, and the respiratory muscles studied: all vary from one worker to another. Further, in some instances where the diaphragm was exposed, cooling of the muscle may have diminished considerably its

sensitivity to the drug (cf. Holmes, Jenden & Taylor, 1947). Finally, the natural stimulus received by the respiratory muscles during curarization is not constant, but changes as asphyxia advances; so that it is not easy to compare an experiment in which single nerve shocks, regularly applied, are used to excite the diaphragm, with one in which natural respiration is retained.

For the same reasons it is uncertain in what way our results on animals are applicable to man, until there is more information about the physiological characteristics of human muscle and of the nervous activity that excites it.

The effects of ether

The different action of ether anaesthesia on the responses to decamethonium and to D-tubocurarine are readily understood if we remember that decamethonium, like acetylcholine, depolarizes the end-plate region, whereas D-tubocurarine renders the end-plate less sensitive to acetylcholine. Ether was found to lessen the sensitivity of cat muscle to decamethonium, to depress the potentiation of the twitch due to decamethonium, to make the action of decamethonium somewhat more like that of curare (in that tetani are no longer well held, and respiratory muscle is more affected), and to intensify the action of D-tubocurarine. Similarly, ether is known to raise the threshold of cat muscle to acetylcholine, and to abolish the repetitive response of the muscle to single nerve shocks after eserine (Simonart & Simonart, 1934; Brown, Dale & Feldberg, 1936); it also causes neuromuscular block to which neostigmine has some antidotal action (Poulsen & Secher, 1949). We may summarize these actions by saying that ether raises the threshold of the motor end-plate to decamethonium equally with the threshold to acetylcholine; ether is bound to affect the neuromuscular blocks due to decamethonium and to D-tubocurarine in different ways.

Both ether and D-tubocurarine thus produce their effects at the neuromuscular junction by making the end-plates less sensitive to depolarizing agents, but it is unlikely that they do so by the same means. The action of D-tubocurarine, like that of similarly acting quaternary salts, is explained by its competition with acetylcholine or decamethonium respectively for the specific receptors in the end-plate region. The action of ether certainly cannot be regarded in this way. There is evidence, however, that it can lessen the permeability of the cell-membrane and increase its electrical impedance (see Höber, 1945, for references). Such changes could well be associated with a resistance to the depolarizing action of acetylcholine and decamethonium, so that the threshold to these substances would rise. Such a mechanism is, of course, quite distinct from the specific curare-acetylcholine antagonism at the end-plate, and analysis would probably reveal significant differences of behaviour.

The behaviour of 'red' and 'white' muscles in neuromuscular block

It is well established that muscles differ in their sensitivity to curare, and similar variations in sensitivity have also been observed with decamethonium in man (Organe, Paton & Zaimis, 1949). But there is less agreement in the literature as to which muscles are more and which less sensitive to the various blocking agents (cf. Bremer & Titeca, 1935; Hartridge & West, 1931; Garry, 1933; Brown & Harvey, 1941). Bremer & Titeca (1935) in a study of the effects of calabash curare on the postural tone of skeletal muscle, found that these were due to a selective action by curare on 'red' muscles. But in a comparison of the sensitivity to curare of gastrocnemius and soleus (excited by single nerve shocks), they could not demonstrate any difference in the direct sensitivity of these muscles to the drug. They attributed the selective action of curare on muscles subserving tone to the fact that these muscles were in constant activity, and produced evidence that in the presence of small doses of curare such activity would be sufficient to cause the appearance of Wedensky inhibition. This explanation is similar to that which we have advanced above, to account for part of the effect of D-tubocurarine on the respiration. But although our experiments on the sensitivity of the muscles themselves have had a different result, the distinction between 'red' and 'white' muscle is useful in understanding our findings with D-tubocurarine and decamethonium.

No physiological or histological distinction between 'red' and 'white' muscle can be drawn that is consistent over the whole musculature (cf. Needham, 1926; Creed, Denny-Brown, Eccles, Liddell & Sherrington, 1932). But for most of the skeletal muscles 'redness' is associated with slow contraction and easy fusion of tetanic stimuli, and 'whiteness' with rapid contraction and fusion only at high frequencies. According to this distinction soleus can readily be classed as a 'red' and tibialis mainly as a 'white' muscle. But the behaviour of tibialis is more complex. Gordon & Holbourn (1949) have shown that its deep fibres are 'redder' and contract more slowly than its superficial fibres. This fact affords a fresh demonstration in a limb muscle of the association of muscle colour with speed of contraction and fusion frequency. Nevertheless, the whole muscle is 'whiter' than soleus and its contraction faster, so that in general it has to be classified as a 'white' muscle.

There appear to be no published data as to the characteristics of respiratory muscle. From our experience of the action of D-tubocurarine in the cat, we would predict that respiratory muscle should resemble soleus rather than tibialis. In fact, we have found that in an animal freed of blood by perfusion with saline, the diaphragm is distinctly 'redder' than tibialis; and the intercostal muscles are 'redder' still, resembling soleus in tint. If the respiratory muscles can thus be classified with soleus rather than with tibialis, then our

pharmacological results with these muscles provide consistent evidence for the selective sensitivity of 'red' muscle to curare.

The muscles examined differ in their sensitivity not only to D-tubocurarine, but also to decamethonium. But, unlike D-tubocurarine, decamethonium was found to be less effective in causing neuromuscular block in those muscles which can be classified as 'red' than in the predominantly 'white' tibialis muscle. There is, in fact, an inverse relationship between the two blocking agents.

This correlation of type of muscle with sensitivity to blocking agents enables us to interpret some details of the results obtained on the tibialis muscle. The observed increase in tetanus/twitch ratio of the muscle during the development of block due to decamethonium, appeared originally a rather puzzling phenomenon; but it can be readily explained in the light of Gordon & Holbourn's observation that tibialis is a compound muscle. For, if the deeper part of the muscle contracts more slowly and fuses tetani more readily than the superficial parts, it ranks nearer to the 'red' type of muscle like soleus, and it should therefore be more resistant to decamethonium than the superficial parts. During an increasing block due to decamethonium, then, the character of the muscle contraction would thus become more and more that of the deeper part, and the tetani would fuse more readily, and therefore the tetanus/twitch ratio must rise. This is precisely what happens.

Inverse relationship between neuromuscular block due to decamethonium and that due to curare

The inverse relationship between the two blocking agents is one of their most striking features. Those circumstances which enhance the activity of curare depress that of decamethonium (ether anaesthesia, the use of 'red' muscle); whereas decamethonium is more active when curare is less so (chloralose anaesthesia, the use of 'white' muscle). It is remarkable that the same situation exists when the sensitivity of various species is compared; the order cat-rabbit-mouse-rat is that in which curare becomes increasingly, and decamethonium decreasingly, effective. A third example is provided by the effect of a previous, small dose of curare; this intensifies the action of a further injection of curare, whereas it may raise two- or three-fold the dose of decamethonium required to produce block (Paton & Zaimis, 1949*b*).

To understand this inverse relationship between the activity of decamethonium and D-tubocurarine, we must realize that the mechanism by which the block is produced is different in the two cases, and that the action of decamethonium, apparently, is closely similar to that of acetylcholine. We know that decamethonium, like acetylcholine, can excite amphibian, avian, and mammalian muscle; both compounds can produce neuromuscular block; and both compounds depolarize the muscle membrane, chiefly at

the end-plate region (Kuffler, 1943; Paton & Zaimis, 1949*b*; Burns *et al.* 1949; Zaimis, 1949; Buttle & Zaimis, 1949). Decamethonium and acetylcholine, by their depolarizing action at the end-plate may, according to the circumstances, either initiate contraction or block transmission from the end-plate to the muscle fibre. If we assume, then, that decamethonium acts at the motor end-plate like acetylcholine, whereas D-tubocurarine opposes the action of acetylcholine, and that the sensitivity of the end-plate to acetylcholine and to decamethonium varies in the same way in different muscles under different conditions, the inverse relationship described becomes explicable. Suppose that the threshold at the end-plate region rises to the stimulating action of acetylcholine, then more decamethonium would be needed to produce block because the end-plate threshold rises similarly to the blocking property of decamethonium; but less D-tubocurarine would be required, because the acetylcholine released at the nerve terminals is now already partly antagonized. On the other hand, a decrease in threshold to acetylcholine renders the end-plate also more susceptible to the paralysing properties of decamethonium, but more curare will then be required to antagonize the enhanced stimulating action of acetylcholine. Whether the difference in sensitivity arises from the comparison of different types of muscle, different species, different anaesthetics, or other conditions, it is bound to affect the neuromuscular block due to decamethonium and D-tubocurarine in opposite ways.

The experiments described in this paper, therefore, support the supposition that decamethonium and acetylcholine act in closely similar ways. Indeed, it is probable that the sensitivity and the reactions of a muscle to decamethonium are an important guide to its sensitivity and reactions to acetylcholine; decamethonium may, therefore, be useful when the instability or other properties of acetylcholine make it impracticable to use this substance itself. The characteristics of the decamethonium block, which we have described and contrasted with those of block due to D-tubocurarine both in this paper and elsewhere, may be taken to represent the typical behaviour of block due to acetylcholine.

SUMMARY

1. In cats anaesthetized with chloralose, decamethonium depresses the nerve-excited twitch of tibialis by 95% before depressing the respiration. But D-tubocurarine depresses the respiration before the twitch is at all affected. Ether anaesthesia increases the respiratory depressant action of both drugs.

2. In unanaesthetized monkeys and cats, the dose of decamethonium required to suspend respiration is 2–2½ times that required to paralyse other movements, whereas with D-tubocurarine it is only 1¼–1½ times as great.

3. D-Tubocurarine and decamethonium do not alter the respiratory discharge down the phrenic nerve during their action in depressing respiration, until asphyxia supervenes, when the discharge is intensified.

4. D-Tubocurarine does not cause any mechanical hindrance to the respiration, such as bronchoconstriction.

5. D-Tubocurarine depresses a tetanus of a muscle excited through its nerve more than it depresses single contractions; but, during neuromuscular block due to decamethonium, the tetanus/twitch ratio of tibialis is maintained, and rises as block deepens.

6. D-Tubocurarine depresses the contractions of soleus excited through its nerve more readily than those of tibialis, and decamethonium tibialis more readily than soleus. The changes of respiratory minute volume follow those of contractions of the soleus fairly closely after both these drugs.

7. D-Tubocurarine acting in the presence of a fully effective dose of prostigmine retains its respiratory depressant action.

8. The diaphragm and intercostals in the saline-perfused animal are 'red' muscles. It is suggested that in the skeletal musculature 'red' muscles are sensitive to curare and resistant to decamethonium, and 'white' muscles vice versa.

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